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19. ABSTRACT (Continue on reverse if necessary and identify by block number) A model of hemorrhagic shock has been developed using rabbits. Rabbits are anesthetized and instrumented for comprehensive hemodynamic monitoring. Blood is withdrawn to obtain a mean arterial pressure of 35 mmHg. Retransfusion with autologous warmed shed blood is performed either 30 or 60 minutes following blood withdrawal. Tissue samples are obtained at various time intervals for determination of antioxidant enzymes activities. Results to-date indicate that superoxide dismutase activity either declines or is unchanged in plasma, myocardium, brain, large and small intestines, liver, skeletal muscle, and lung. In contrast, catalase activity is induced with substantial increases in catalase activity in each of the aforementioned tissues. These results may have important implications for understanding the pathophysiology of hemorrhagic shock and consequently, in the design of therapeutic strategies for resuscitation from hemorrhagic shock.					
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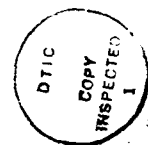
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EFFECTS OF HEMORRHAGIC SHOCK ON ANTIOXIDANT ENZYME ACTIVITIES

ANNUAL REPORT: July 1, 1988 to June 30, 1989

Prepared for the Office of Naval Research
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INTRODUCTION

Hemorrhagic shock from trauma or other causes continues to be a major cause of morbidity and mortality, and despite extensive research, the mechanisms of tissue damage remain poorly characterized. The mainstay of treatment is rapid cessation of hemorrhage and restoration of an adequate circulating blood volume with whole blood, colloid and/or crystalloid solutions. Despite these measures, many individuals will eventually succumb in a downward spiral of metabolic, neuroendocrine, and cardiovascular responses (1-3).

Substantial evidence has recently been advanced that supports the concept of "reperfusion injury" following an ischemic insult (4-6). According to this theory, during the period of reperfusion, additional tissue injury occurs mediated primarily by oxygen-derived free radicals (superoxide and hydroxyl radicals). The best documented source of oxygen derived free radicals is the enzyme xanthine oxidase (7); the formation of which from the enzyme xanthine dehydrogenase is known to occur in certain tissues during the period of ischemia (8). With restoration of normal microcirculatory flow and supply of molecular oxygen, superoxide radical is generated in great quantity. In some models, natural antioxidant enzyme activities are reduced during the period of ischemia, further contributing to reperfusion injury (8).

Most of the work done in this area has been with tissue or isolated organ preparations. Because severe hemorrhagic shock represents "total body ischemia", then fluid resuscitation for hemorrhagic shock could be analogous to "reperfusion injury" mediated by oxygen-derived free radicals. This paper presents data on the antioxidant enzyme systems superoxide dismutase and catalase in a whole animal model of severe hemorrhagic shock and resuscitation.

METHODS

Mature male New Zealand white rabbits were anesthetized with pentobarbital sodium (50 mg/kg i.v.) and then supplemented as necessary throughout the experiment. A tracheostomy was performed immediately and mechanical ventilation was instituted. The animals were kept on a warming blanket and heating lamps were used to maintain a normal rectal temperature (recorded continuously). Continuous ECG monitoring was done throughout the experiment. A femoral cutdown was then

performed for the introduction of catheters into the descending aorta and inferior vena cava via the femoral artery and vein. The chest was opened in the midline so that catheters could be placed directly into the left and right atria, left ventricle, and main pulmonary artery (via a puncture in the right ventricular outflow tract). An ultrasonic flow probe (Transonics Systems) was fitted onto the ascending aorta. These surgical procedures required 30 to 40 minutes to complete. This preparation allowed for continuous monitoring of heart rate and pressures in the descending aorta, atria, main pulmonary artery, and left ventricle. Ascending aortic flow was monitored continuously and was used to determine stroke volume and cardiac output. The maximum rate of left ventricular pressure development (LV dP/dt) was derived from the left ventricular pressure curve. Systemic and pulmonary vascular resistances were calculated from the pressure and flow data. Arterial blood gas measurements were determined every 20 to 30 minutes, and the ventilator rate/volume was adjusted to maintain a normal $p\text{CO}_2$. The animals were ventilated with room air only (no supplemental oxygen).

Following surgical instrumentation, the animals were allowed to stabilize for 30 minutes. Control hemodynamic parameters and blood gases were recorded at the end of this 30 minute period. The groups and experimental protocol are described in Table 1. The first and second experimental groups had blood withdrawn (into a warmed reservoir that contained 100 U/kg of heparin) to reduce the mean arterial pressure to 35 mmHg initially. Additional blood was not withdrawn during the shock period and the intrinsic physiologic compensatory mechanisms were allowed to attempt to compensate for this acute blood loss. One experimental group was maintained for 30 minutes (30 min shock group) and another for 60 minutes (60 min shock group) prior to retransfusion. Hemodynamic parameters and blood gases were again recorded at the end of these "shock" periods. These two experimental groups were then resuscitated from hemorrhagic shock by reinfusing (over 2 to 3 minutes) the warmed autologous shed blood. Following an additional 30 minute period (resuscitation period), hemodynamic parameters and arterial blood gases were recorded and the animals were sacrificed by an overdose of pentobarbital. Two control groups underwent identical surgical preparation and the 30 minute stabilization period, but were not bled or resuscitated. The first control group was time-matched to correspond to the 30 minute shock experimental group; the

second control group was time-matched to correspond to the 60 minute shock experimental group.

Organ samples were taken immediately after the animal was sacrificed. Tissues sampled included the heart (ventricular myocardium), lung, liver, small intestine, large intestine, skeletal muscle, brain, and blood. These tissues were placed in vials and kept on ice. One gram of each tissue was homogenized in 10 ml of ice cold buffer at pH 7.8. The homogenization buffer contained 50 mM potassium phosphate and 0.1 mM EDTA. A Polytron set at position 5 was used to homogenize the tissue for 3 thirty second bursts. The homogenate was then centrifuged at 14,000 X g for 15 minutes at 4°C. The pellet was discarded and a sample of the supernatant was used for the assays. Blood (10 ml) was centrifuged and an aliquot of plasma was used for the assay.

Superoxide dismutase assay

This assay was performed using a cocktail (pH 7.8) consisting of 50mM potassium phosphate with 5×10^{-5} M xanthine, 10^{-5} M cytochrome-c, and 0.1 mM EDTA. Purified bovine xanthine oxidase was used to generate superoxide anion. The total assay volume was 2 ml. Tissue samples were assayed for ability to inhibit the superoxide-induced reduction of cytochrome-c (monitored spectrophotometrically at 550 nm). SOD activity was calculated based on the definition that one unit of activity results in a 50% inhibition of cytochrome-c reduction under these conditions.

Catalase assay

Tissues were prepared in the manner described above. The buffer contained 50 mM potassium phosphate and 2×10^{-2} M hydrogen peroxide (pH 7.0). Known volumes of tissue sample supernatant were added to 1 ml of this hydrogen peroxide solution and absorbance was monitored at 240 nm. Catalase activity was determined by measuring the rate of decomposition of hydrogen peroxide.

RESULTS

Hemodynamic data

The hemodynamic data are presented in Figures 1 through 8. Results are presented as the mean \pm 1 S.D. Because both control groups were treated identically (one group was followed for 90 minutes following surgery and the other for 120 minutes), the control data were combined. There was a total of 7 control animals; 4 in the 30 minute time-matched control group and 3 in the 60 minute time-

matched control group. The 30 minute shock group contains 3 animals and the 60 minute shock group contains 5 animals.

Figure 1 illustrates the effect of hemorrhagic shock on arterial pH. There appears to be a significant drop in both the 30 min and 60 min experimental groups as compared to control. The pH in the 30 minute shock group continues to fall after resuscitation, while it appears to stabilize or improve slightly in the 60 minute shock group. Figure 2 shows that the heart rate for animals in the 30 minute shock group generally increased or remained the same as controls, but in the 60 minute shock group the rate appears to fall initially and gradually rises during the later half of shock and 30 minutes into resuscitation.

Mean aortic blood pressure, as shown in Figure 3, demonstrated a marked reduction in both experimental groups, as expected from the experimental design. Mean pulmonary artery pressure (fig 4) dropped slightly in both experimental groups during shock. Figure 5 shows that cardiac output was reduced in the 60 minute shock group but not in the 30 minute shock group. The reason(s) for this discrepancy between experimental groups is not clear at the present time because both experimental groups were to be treated similarly through the first 30 minutes of shock. The drop in cardiac output in the 60 minute shock group was due to both a reduction in heart rate (Figure 2) and stroke volume. Left ventricular dP/dt (Figure 6) decreased in both shock groups and recovered during resuscitation. The fall in systemic vascular resistance (Figure 7) in the 30 minute shock group is attributed to the lack of decrease in cardiac output during the shock period. Systemic vascular resistance did not appear to change significantly in the 60 minute shock group. Pulmonary vascular resistance (Figure 8) increased slightly during the initial phase of shock in the 60 minute shock group, followed by a subsequent decline toward baseline.

Superoxide dismutase

Table 2 summarizes the results obtained for each group. Figures 9 - 11 graphically illustrate that SOD activity in the liver, small intestine, and large intestine was slightly reduced or unchanged in the shocked experimental groups as compared to control values. However, SOD activity in the plasma (Figure 12) was significantly reduced in the 30 minute shock group and SOD activity could not be detected in plasma from animals in the 60 minute shock group.

Catalase

Figures 13 - 20 demonstrate that catalase activity increased in all tissues during hemorrhagic shock compared to the control group. These responses appeared to be related to the duration of shock, in that there was consistently an additional increase in catalase activity in the 60 minute shock group compared to the 30 minute shock group. The tissues with the highest enzyme activity include the heart, lung, liver, and small intestine.

CONCLUSIONS

Previous studies have provided indirect evidence to support the hypothesis that resuscitation from hemorrhagic shock results in substantial reperfusion injury due to cytotoxic oxygen-derived free radicals. Crowell (9), using a canine model of hemorrhagic shock, showed an improved overall survival rate in dogs that received allopurinol (an inhibitor of xanthine oxidase). Another canine model of hemorrhagic shock evaluated the effect of intravenous superoxide dismutase (10). In that study, transient improvement was noted, but overall survival was not affected. However, the administration of SOD in this experiment was brief and exogenously administered SOD is known to have a short half-life in the plasma.

Our study employed a rabbit model of hemorrhagic shock to characterize tissue activities of two critically important endogenous antioxidant enzymes: superoxide dismutase and catalase. Using this model, the 60 minute shock group experienced more severe hemorrhagic shock based upon the hemodynamic and pH data shown. In this group, SOD activity in the liver, small intestine, and large intestine (organs which are rich sources of xanthine oxidase) remained essentially unchanged compared to the control group. However, the activity of SOD in the plasma was markedly reduced. The importance of diminished SOD activity in the plasma following hemorrhagic shock and resuscitation remains to be defined. In contrast, catalase activity appears to be induced during shock and resuscitation. This effect was time dependent and was observed in the plasma and in every organ sampled. The mechanism(s) to account for this increase in catalase activity are unclear at present. However, a recent study of heat-shocked rat hearts subjected to ischemia and reperfusion

suggested that catalase activity was induced in the myocardium following this form of stress/injury (11). Conceivably, stress from hemorrhagic shock results in a global induction of catalase activity.

In summary, our results provide characterization of the hemodynamic responses to acute blood withdrawal in the rabbit. These studies indicate that the rabbit appears to be a useful and suitable model to study various aspects of hemorrhagic shock. Furthermore, preliminary data suggest that SOD activity declines in the plasma after resuscitation from hemorrhagic shock. Tissue SOD activity remains essentially unchanged. Catalase activity appears to be induced by shock/retransfusion and activity increases in virtually every tissue examined. These observations may have important implications in the design of therapeutic strategies for the management of hemorrhagic shock.

REFERENCES

1. Runciman WB, Skowronski GA: Pathophysiology of hemorrhagic shock. *Anesth Intensive Care*. 12:193-205, 1984.
2. Elboim CM: Hemorrhagic shock and trauma. *Comp Ther* 11:6-12, 1985.
3. Wilson RF: Science and shock: A clinical perspective. *Ann Emerg Med* 14:714-723, 1985.
4. McCord JM: Oxygen-derived free radicals in post-ischemic tissue injury. *N Engl J Med* 312:159-163, 1985.
5. Weisfeldt ML: Reperfusion and reperfusion injury. *Clin Res* 35:13-20, 1987.
6. Nayler WG, Elz JS: Reperfusion injury: laboratory artifact or clinical dilemma? *Circulation* 74: 215-221, 1986.
7. Parks DA, Granger DN: Xanthine oxidase: biochemistry, distribution, and physiology. *Acta Physiol Scand Suppl.* 548:87-89, 1986.
8. Weiss SJ: Oxygen, ischemia, and inflammation. *Acta Physiol Scand* 548: 9-37, 1986.
9. Crowell JW, Jones CE, Smith EE: Effects of allopurinol on hemorrhagic shock. *Am J Physiol* 216(4): 744-748, 1969.
10. Schoenberg MH, Younes M, Muhl E, Haglund U, Sellin D, Schildberg FW: Free radical involvement in ischemic damage of the small intestine. In: Greenwald RA, Cohen G(eds.) *Oxy Radicals and their scavenger systems: Vol II. Cellular and medical aspects*. New York, Elsevier Science Publishing Co. ,Inc. pp 154-158, 1983.
11. Currie RW, Karmazyn M, Kloc M, Mailer K: Heat-shock response is associated with enhanced postischemic ventricular recovery. *Circ Res* 63: 543-549, 1988.

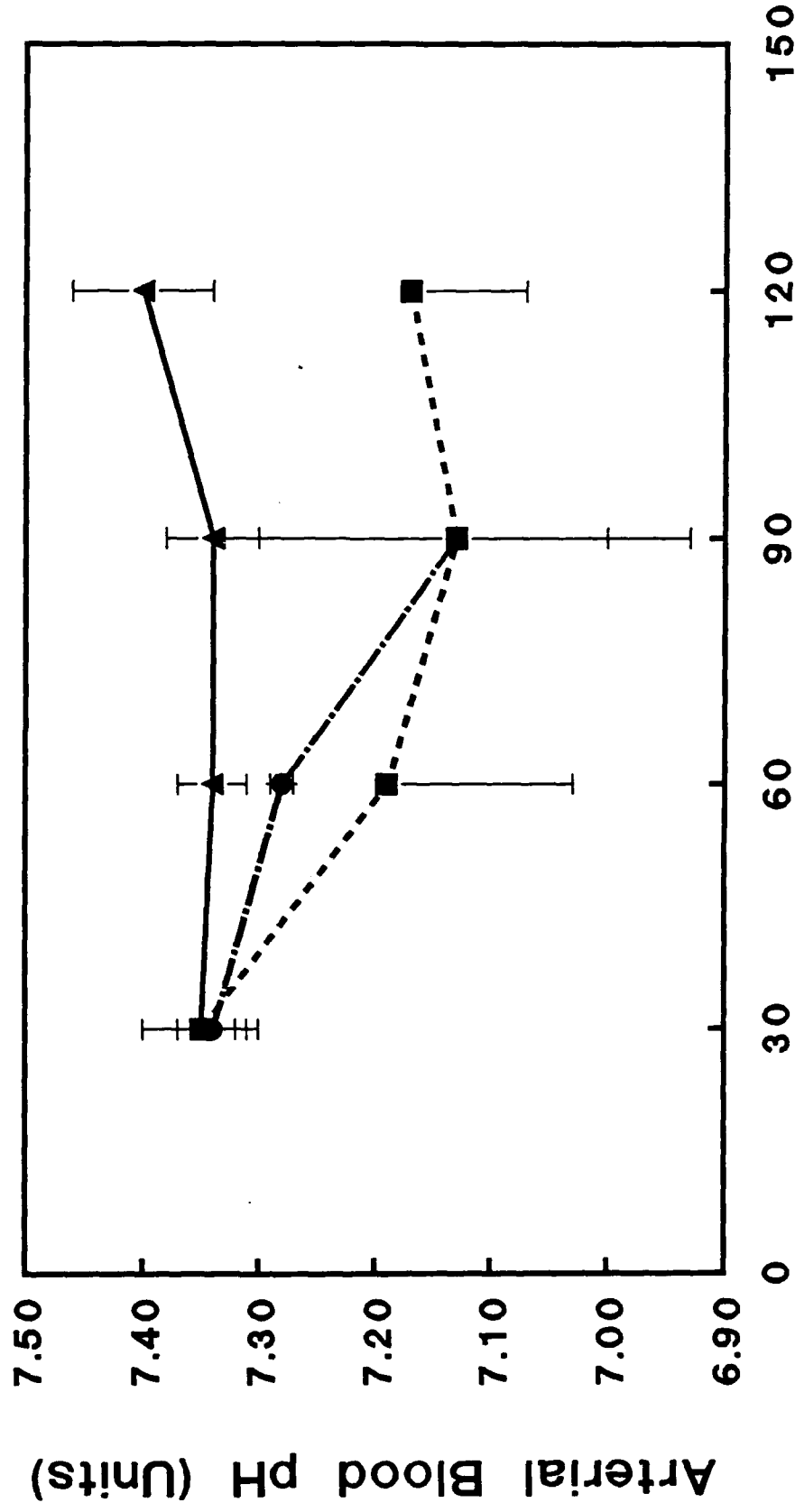
Table 1 Description of the
experimental and control groups

30 minute shock group	Surgery	30 minutes stabilization	30 minutes shock	30 minutes resuscitation	sacrifice
30 minute control group	Surgery	90 minutes stabilization			sacrifice
60 minute shock group	Surgery	30 minutes stabilization	60 minutes shock	30 minutes resuscitation	sacrifice
60 minute control group	Surgery	120 minutes stabilization			sacrifice
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Hemorrhagic Shock

Arterial pH

—▲— Control 30 min —●— Shock 30 min - -■- - Shock 60 min

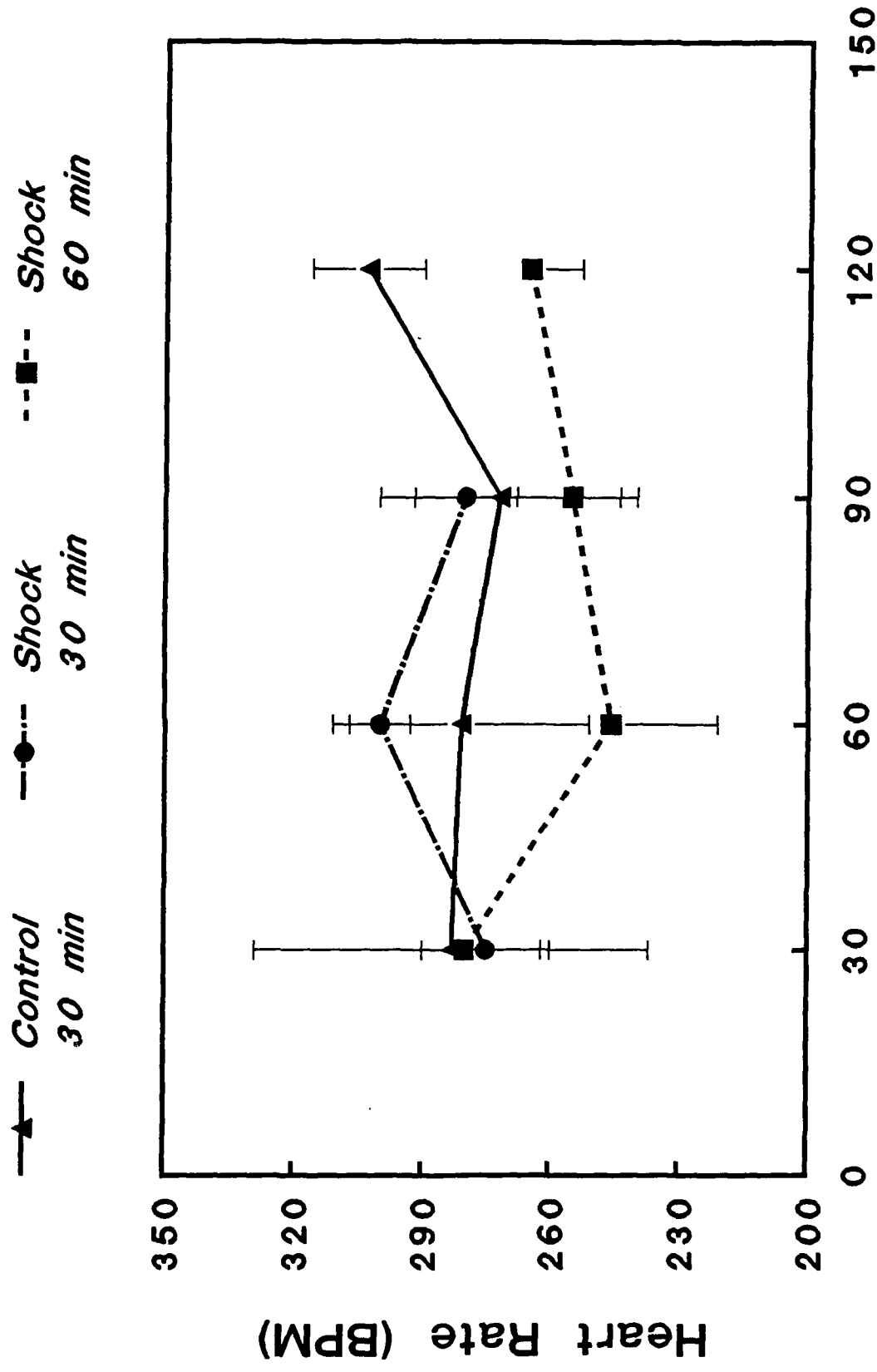


Time After Instrumentation (Minutes)

Figure 1

Hemorrhagic Shock

Heart Rate



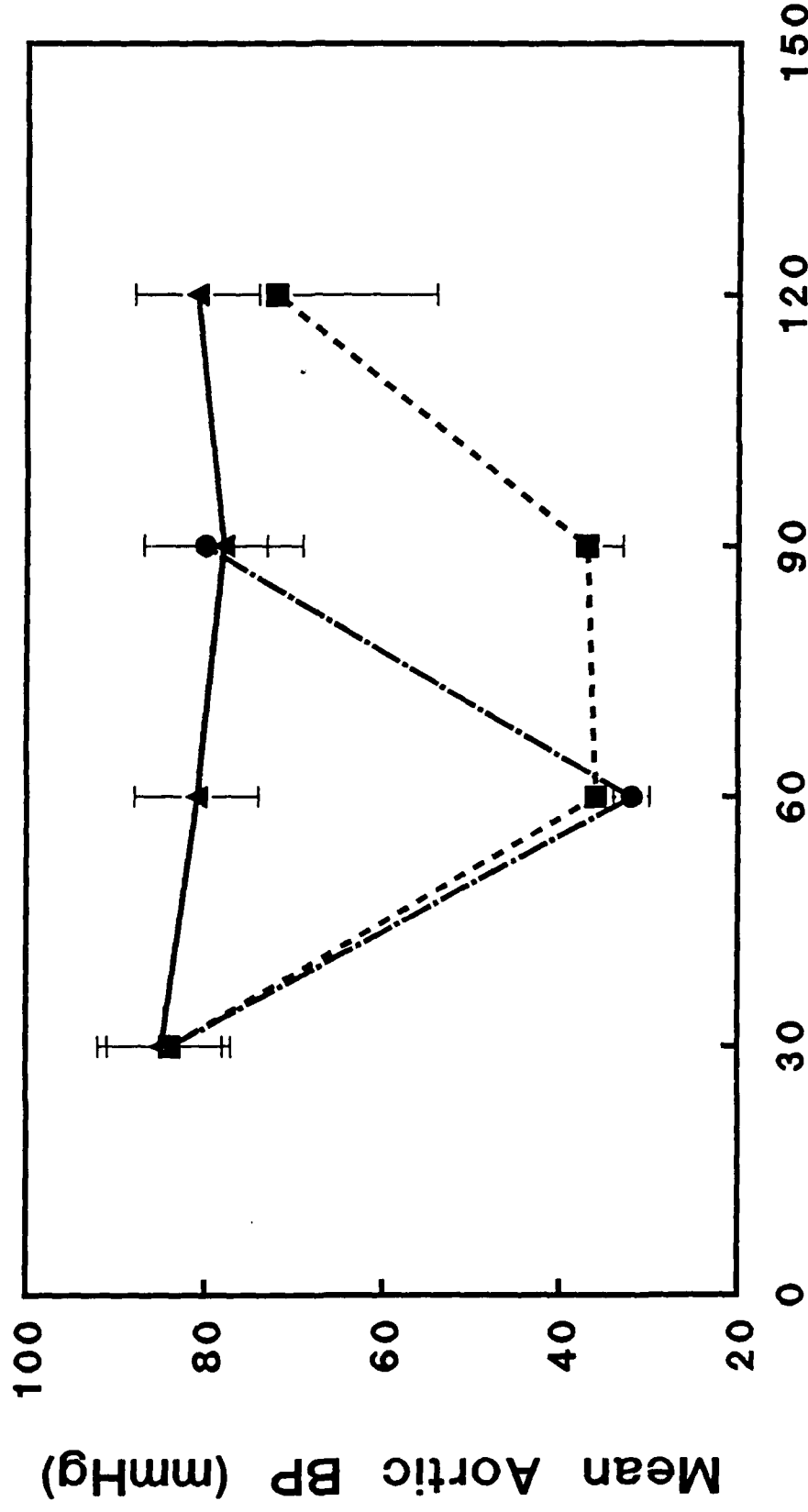
Time After Instrumentation (Minutes)

Figure 2

Hemorrhagic Shock

Mean Aortic Blood Pressure

—▲— Control 30 min —●— Shock 30 min - -■- - Shock 60 min



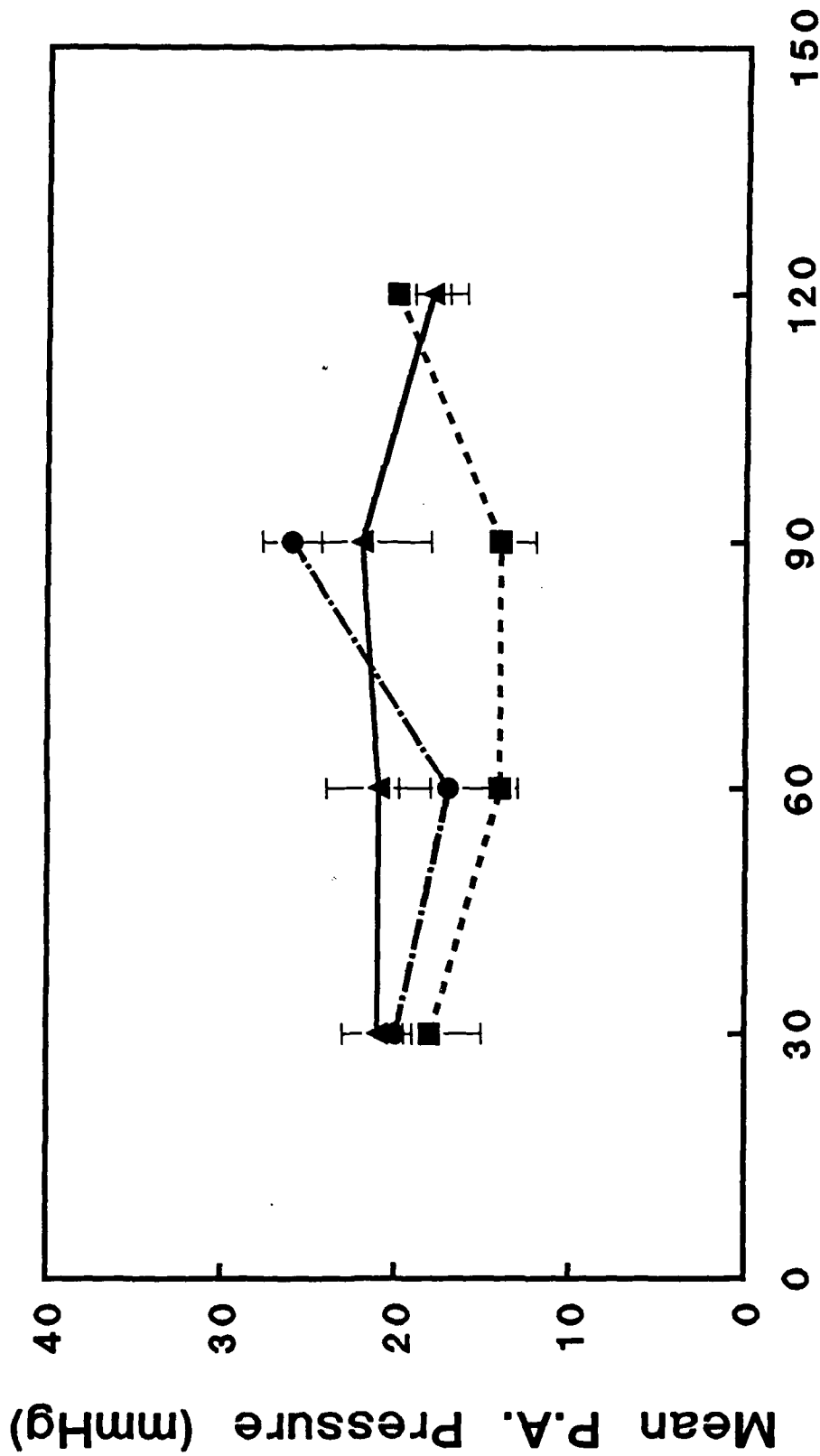
Time After Instrumentation (Minutes)

Figure 3

Hemorrhagic Shock

Mean Pulmonary Artery Pressure

—▲— Control 30 min -●- Shock 30 min -■- Shock 60 min

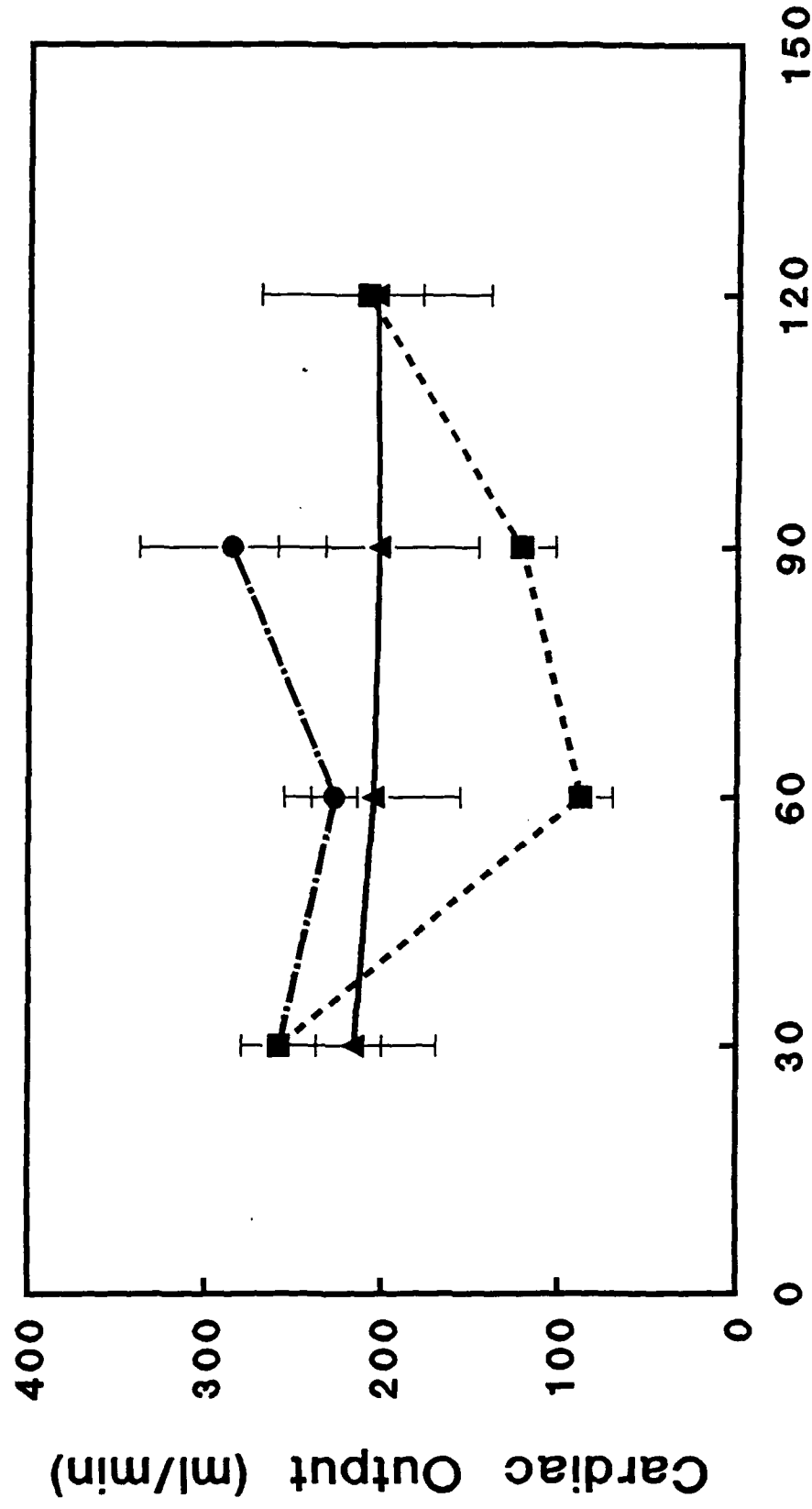


Time After Instrumentation (Minutes)
Figure 4

Hemorrhagic Shock

Cardiac Output

—▲— Control 30 min -●- Shock 30 min -■- Shock 60 min



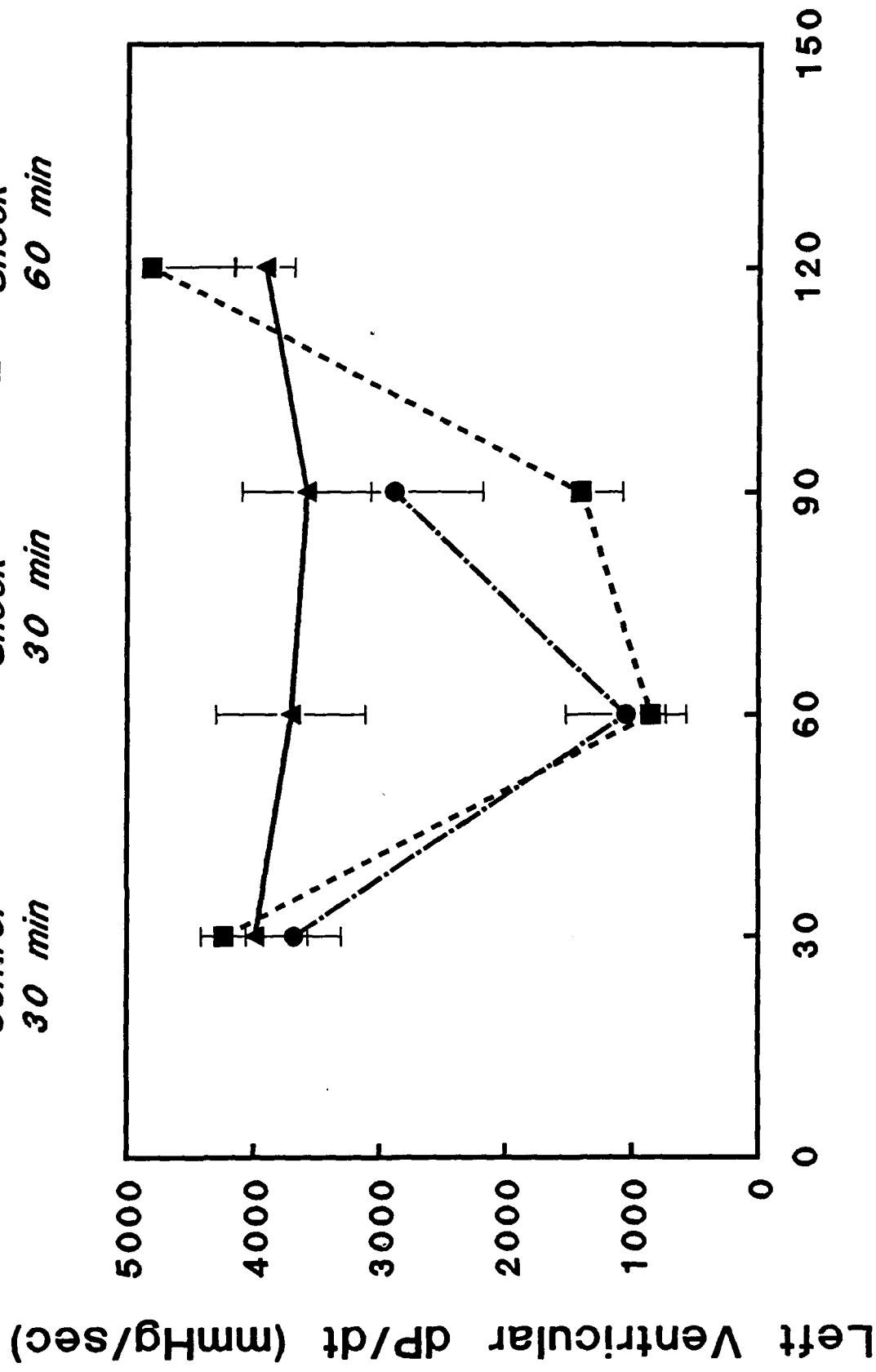
Time After Instrumentation (Minutes)

Figure 5

Hemorrhagic Shock

Left Ventricular dP/dt

—▲— Control 30 min -●- Shock 30 min -■- Shock 60 min



Time After Instrumentation (Minutes)

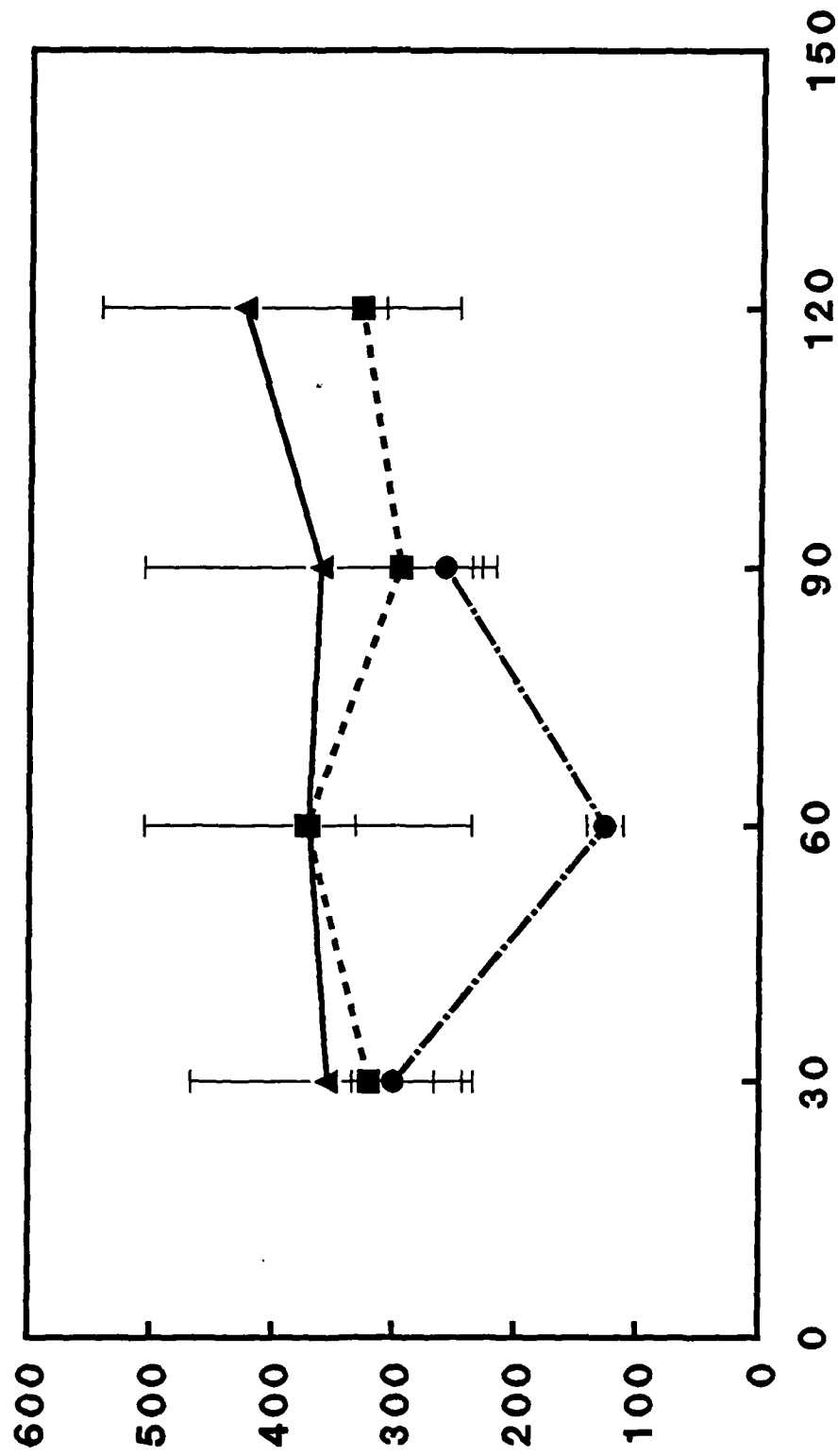
Figure 6

Hemorrhagic Shock

Systemic Vascular Resistance

—▲— Control 30 min —●— Shock 30 min - -■- - Shock 60 min

Systemic Resistance (mmHg/ml/min)



Time After Instrumentation (Minutes)
Figure 7

Hemorrhagic Shock

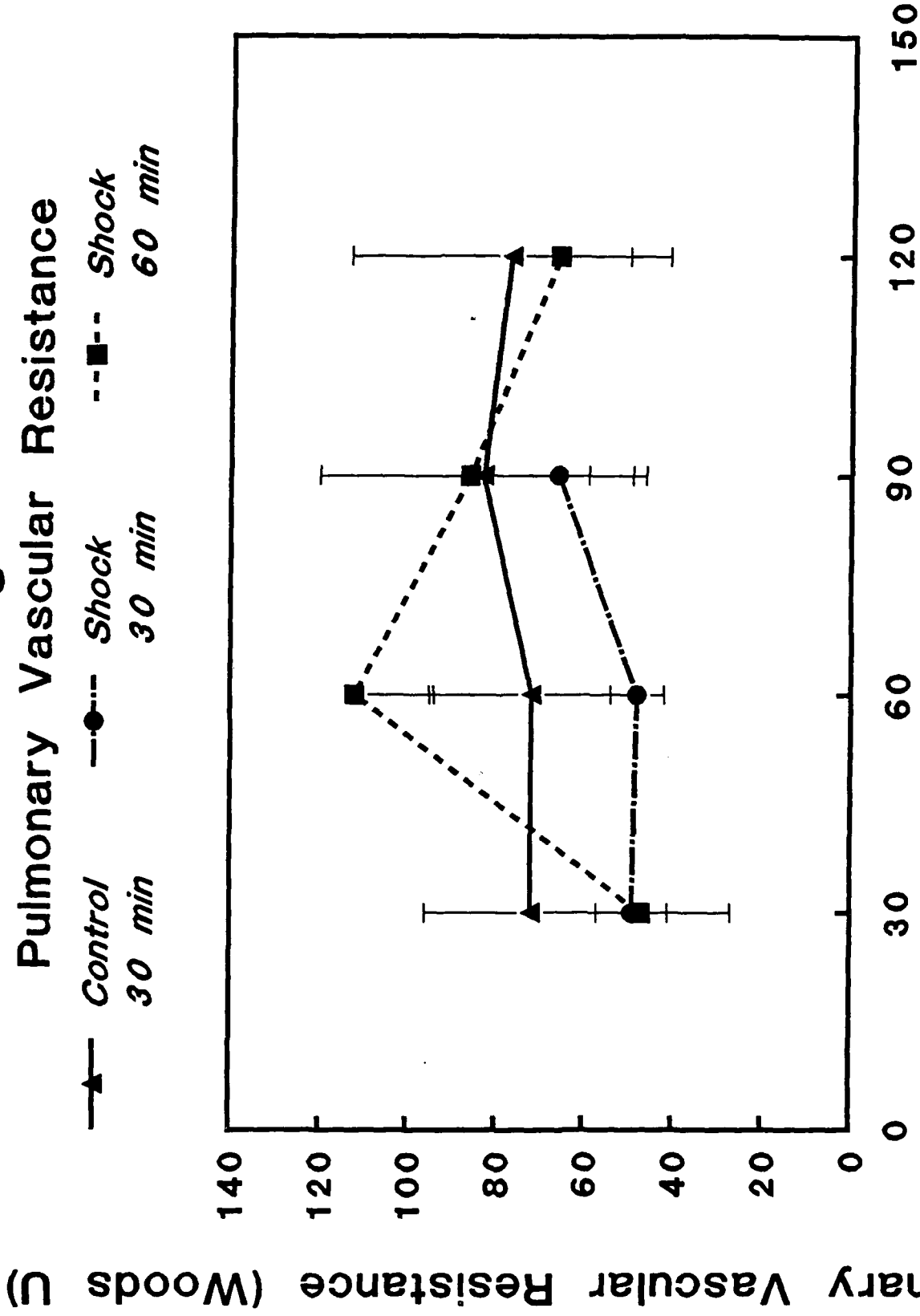


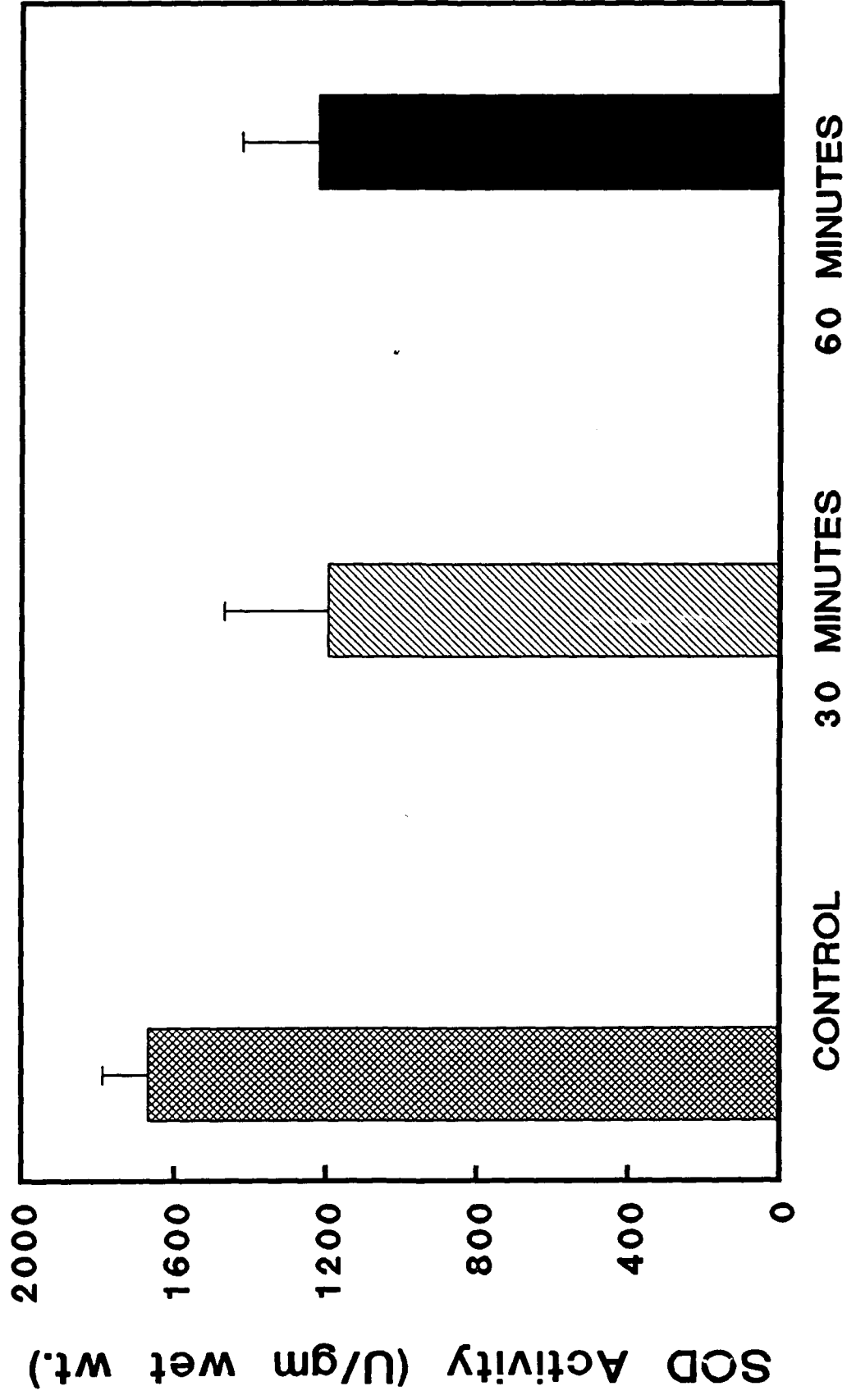
Figure 8

Table 2: Superoxide Dismutase Activity
U/gm tissue wet weight
plasma expressed as U/ml

	30 minute control group n=4	30 minute shock group n=3	60 minute control group n=2	60 minute shock group n=5
Heart	844 +/- 147	644 +/- 64	1632	1210 +/- 353
Liver	1668 +/- 120	1195 +/- 274	2287	1222 +/- 200
Lung	649 +/- 125	854 +/- 216	706	1053 +/- 356
Small Intestine	433 +/- 97	436 +/- 160	411	342 +/- 98
Large Intestine	449 +/- 150	311 +/- 112	339	324 +/- 58
Skeletal Muscle	533 +/- 129	141 +/- 103	264	349 +/- 9
Brain	980 +/- 219	786 +/- 213	1208	-
Plasma	23 +/- 4	10 +/- 2	16	0

Results are expressed as mean +/- 1 S.D.

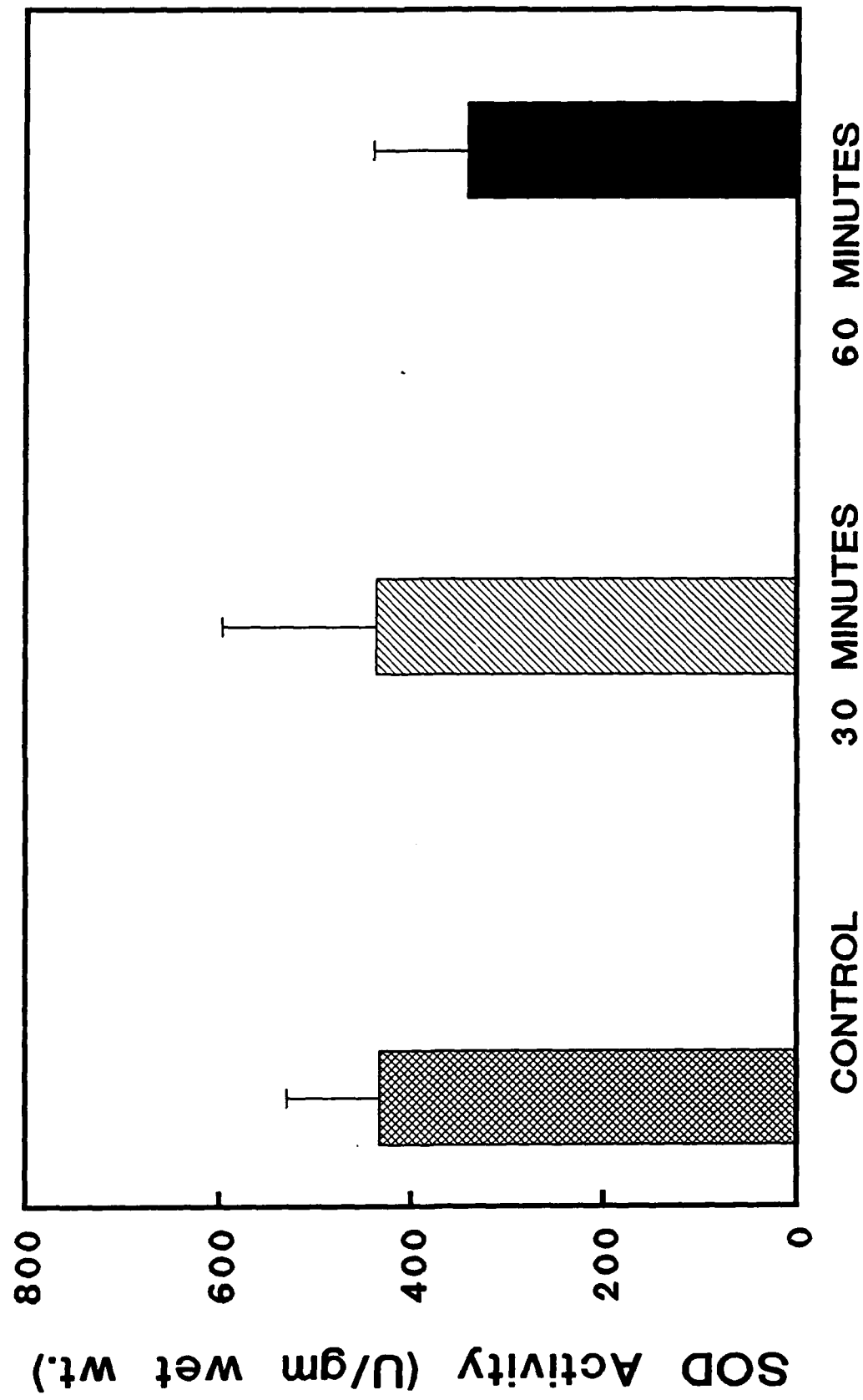
SOD Activity Liver



Group

Figure 9

SOD Activity Small Intestine



Group
Figure 10

SOD Activity Large Intestine

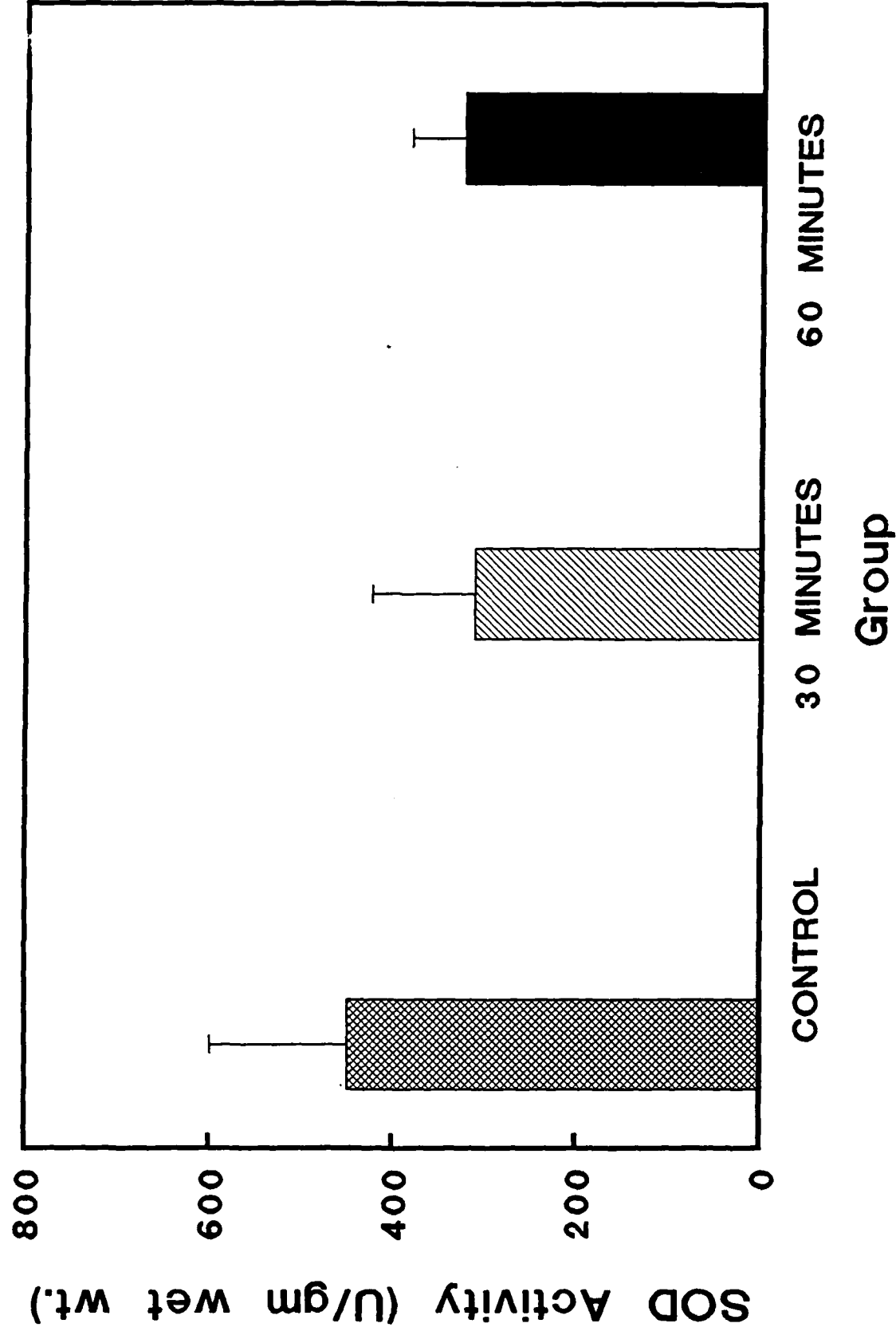


Figure 11

SOD Activity Plasma

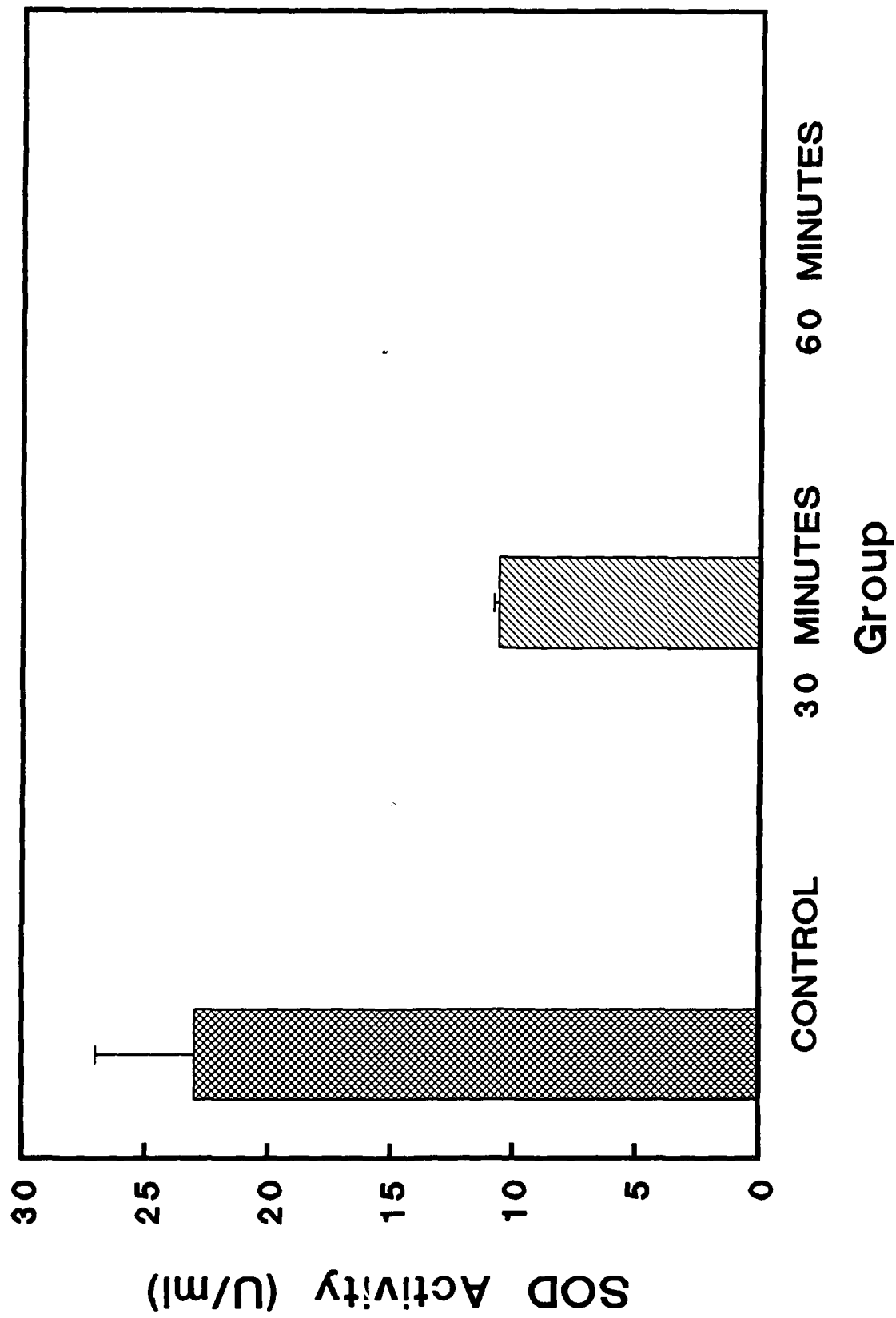


Figure 12

Catalase Activity Heart

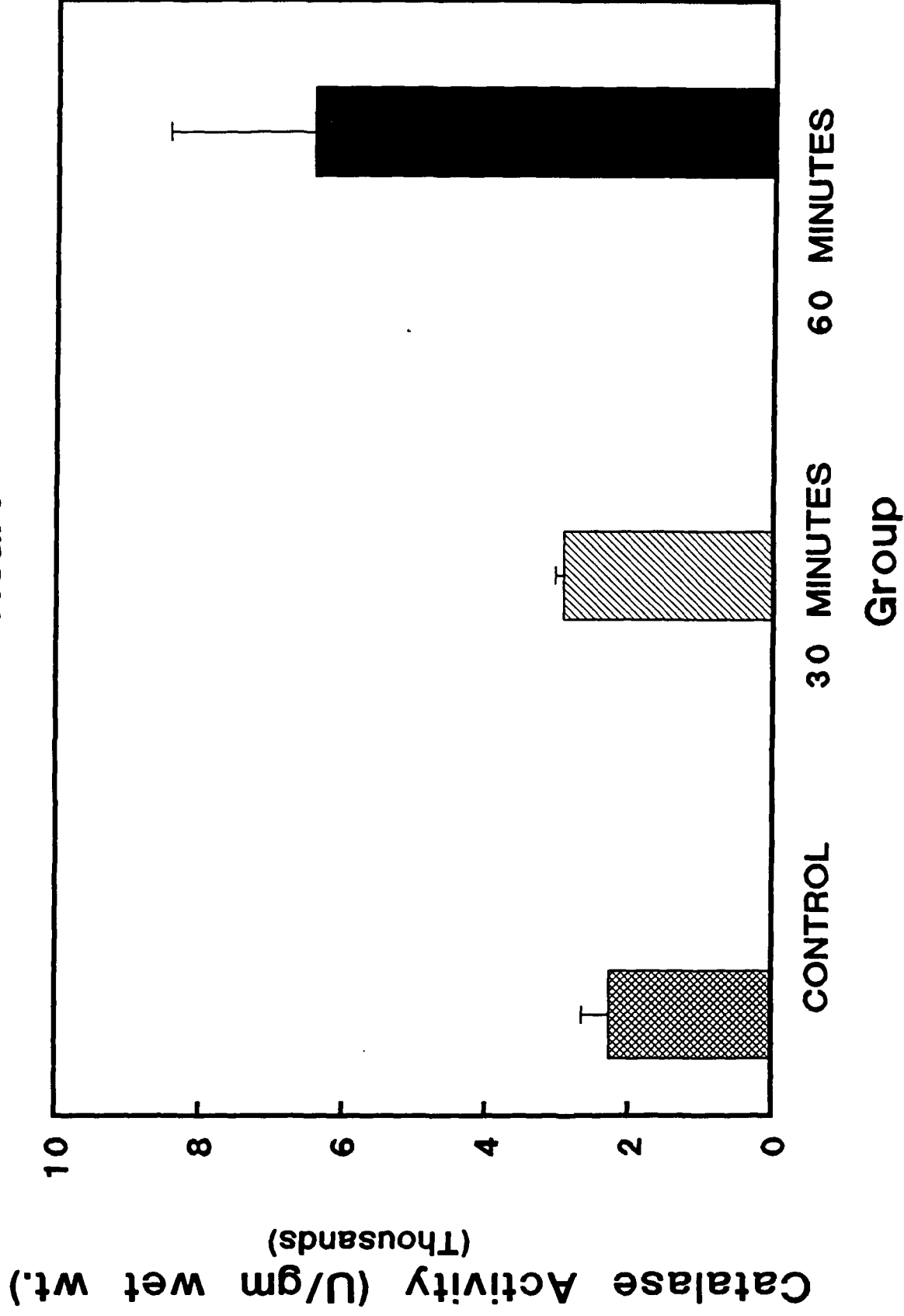


Figure 13

Catalase Activity Lung

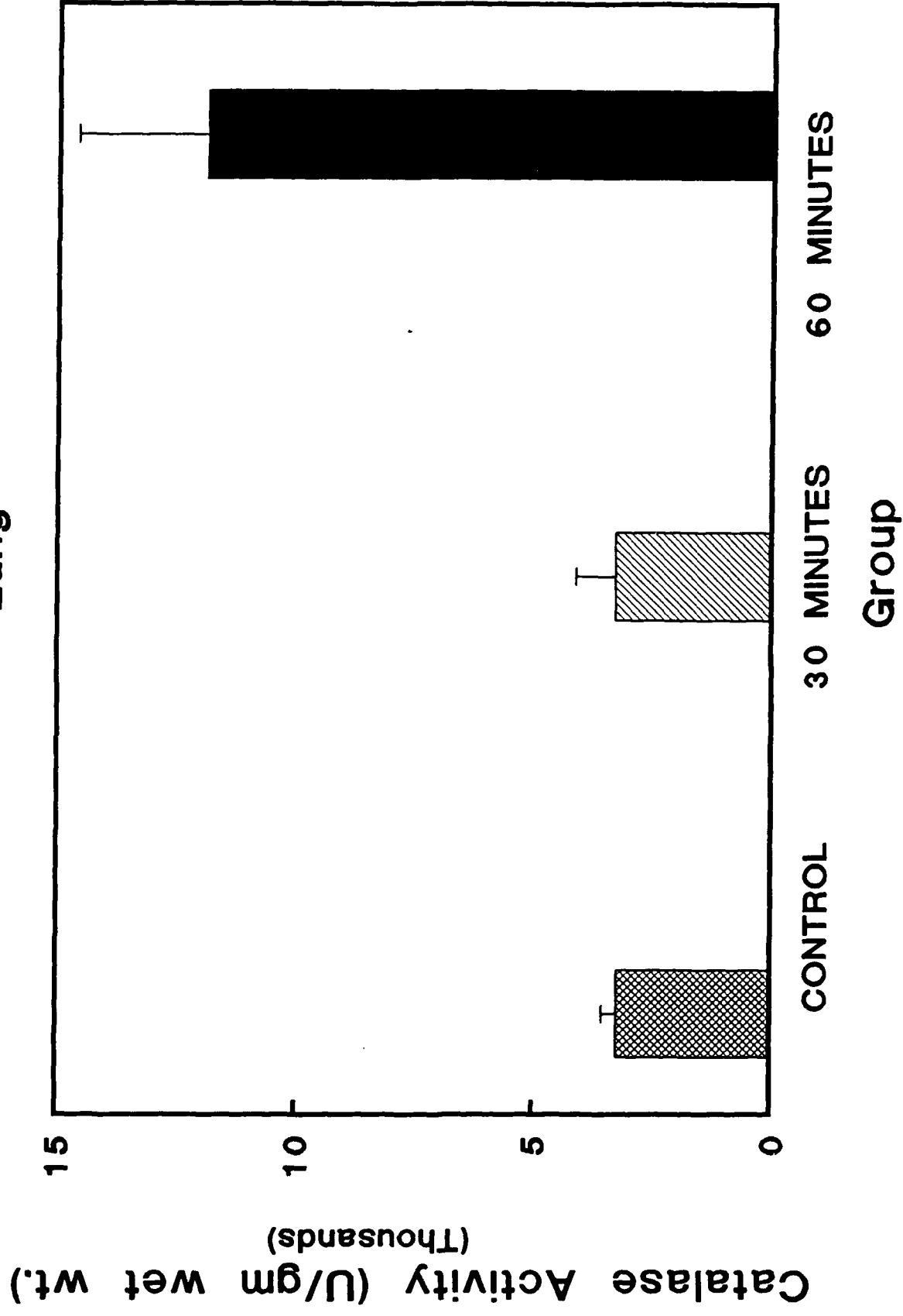


Figure 14

Catalase Activity Liver

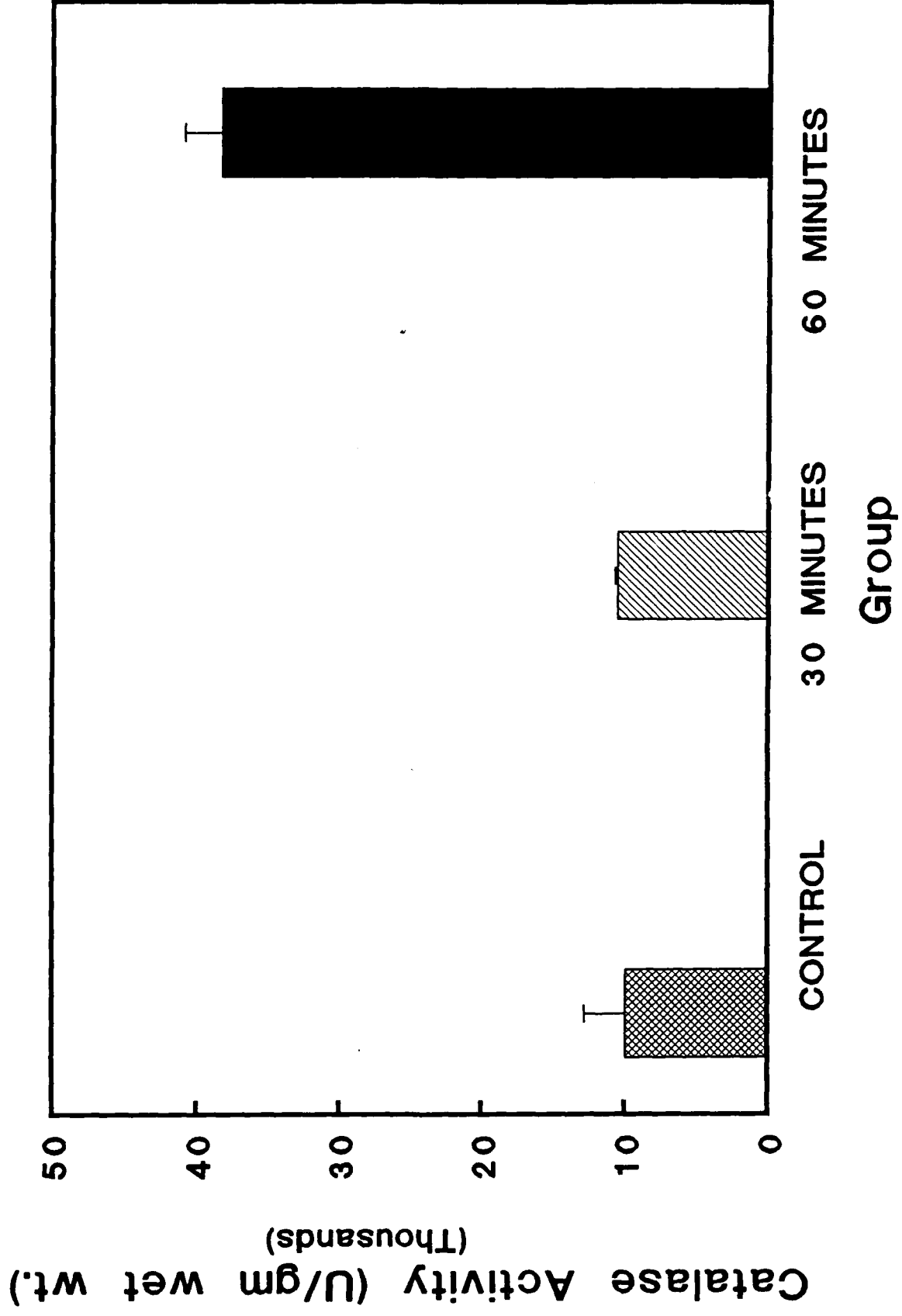


Figure 15

Catalase Activity Small Intestine

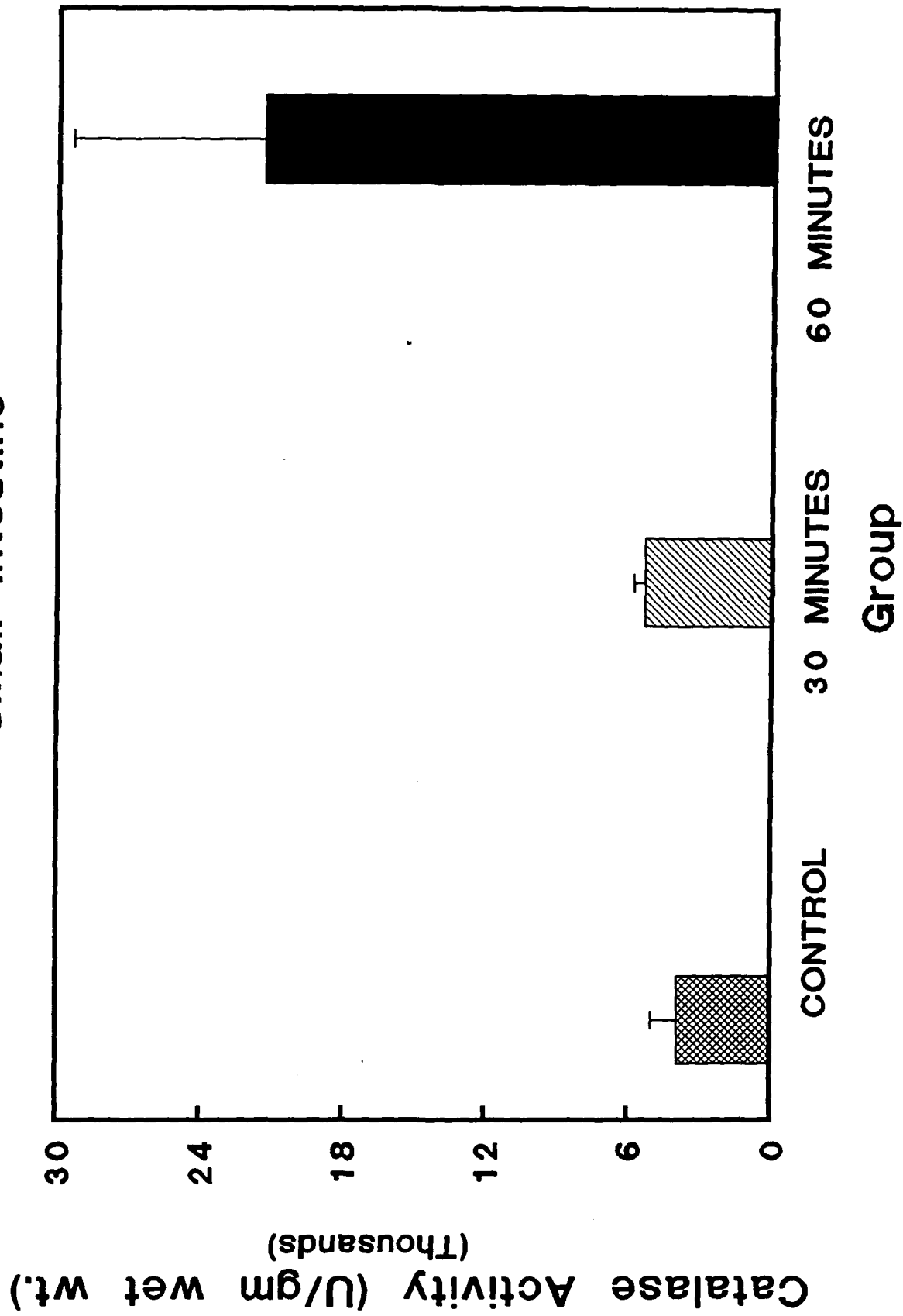


Figure 16

Catalase Activity Large Intestine

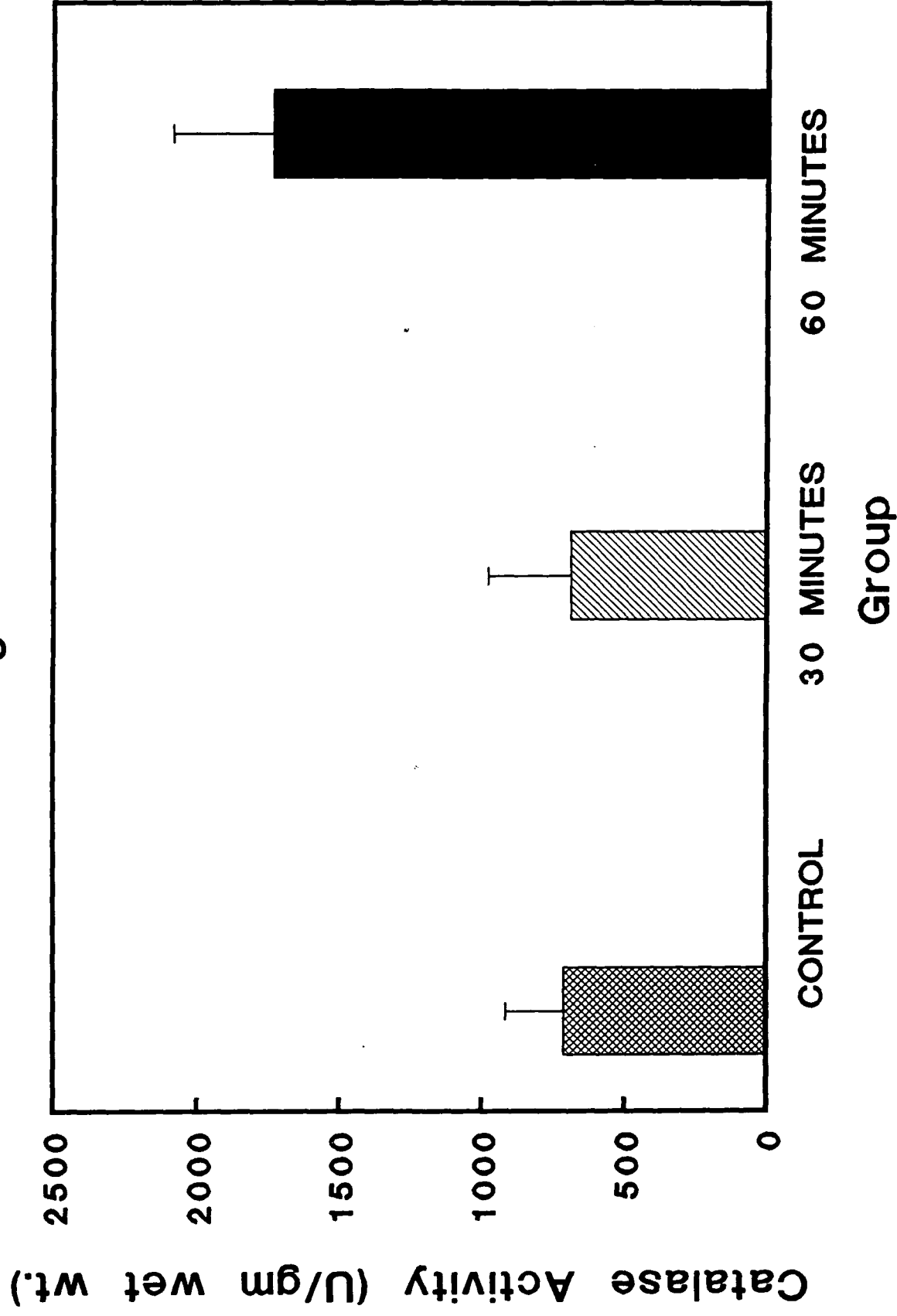


Figure 17

Catalase Activity Skeletal Muscle

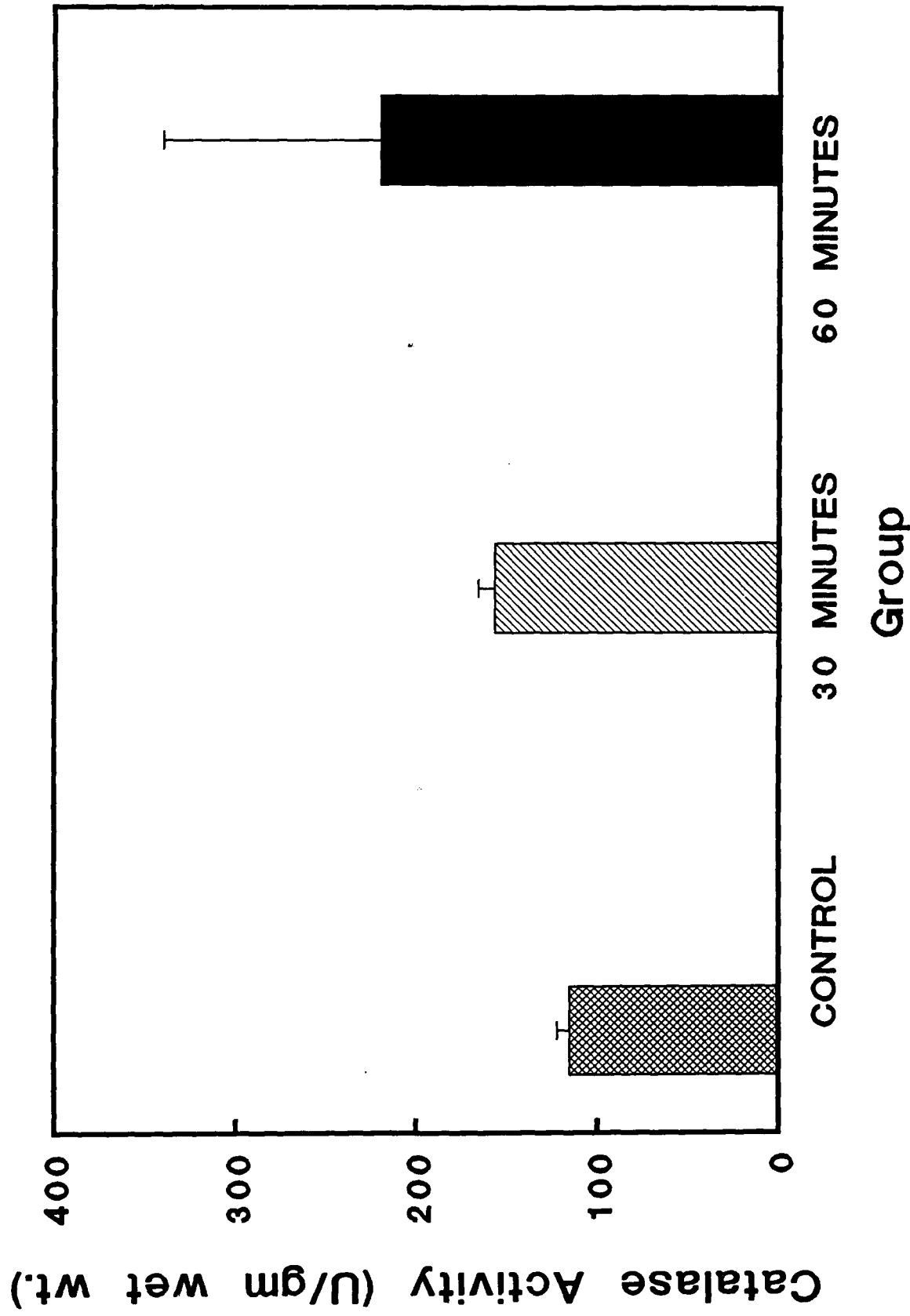


Figure 18

Catalase Activity Brain

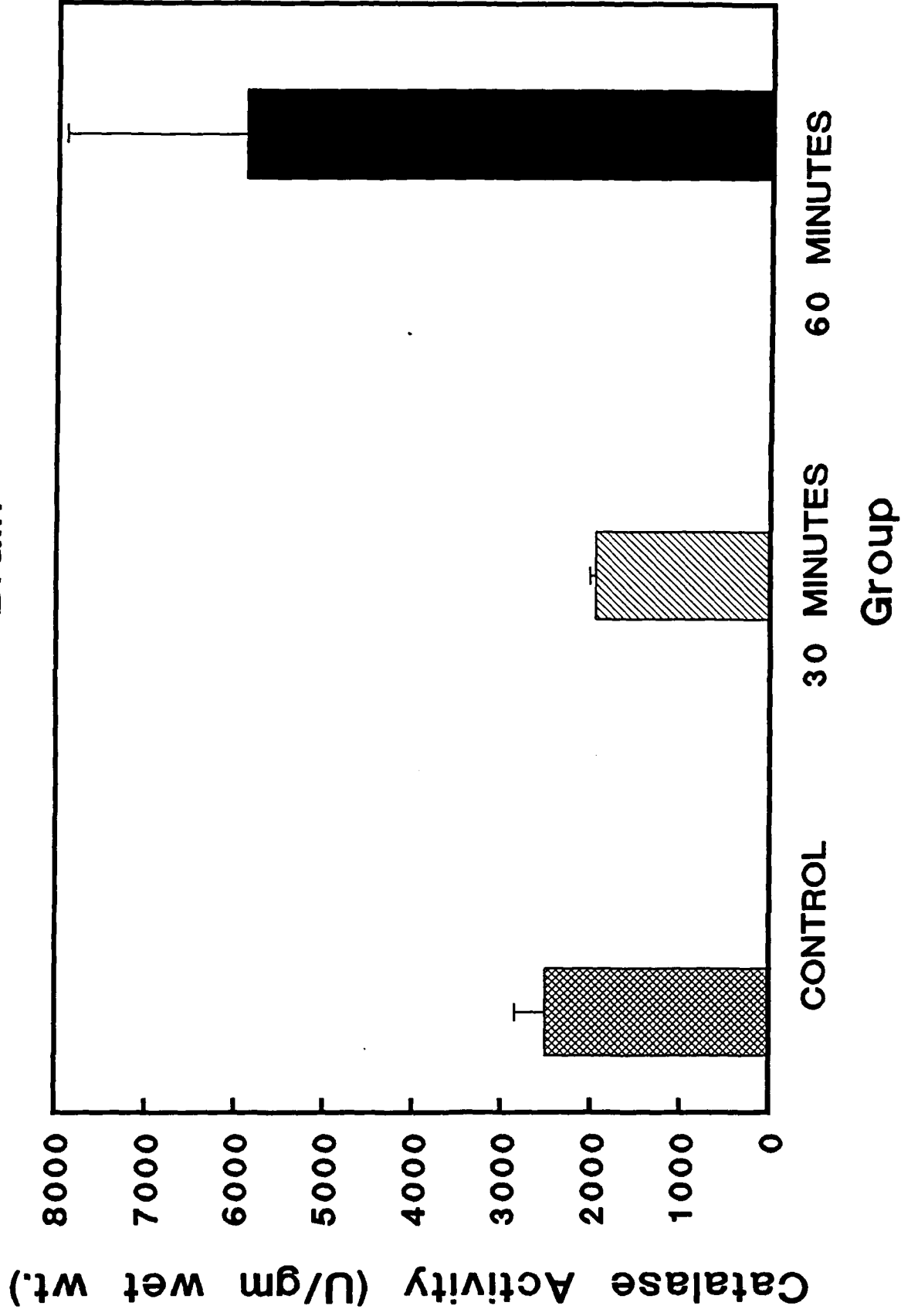


Figure 19

Catalase Activity Plasma

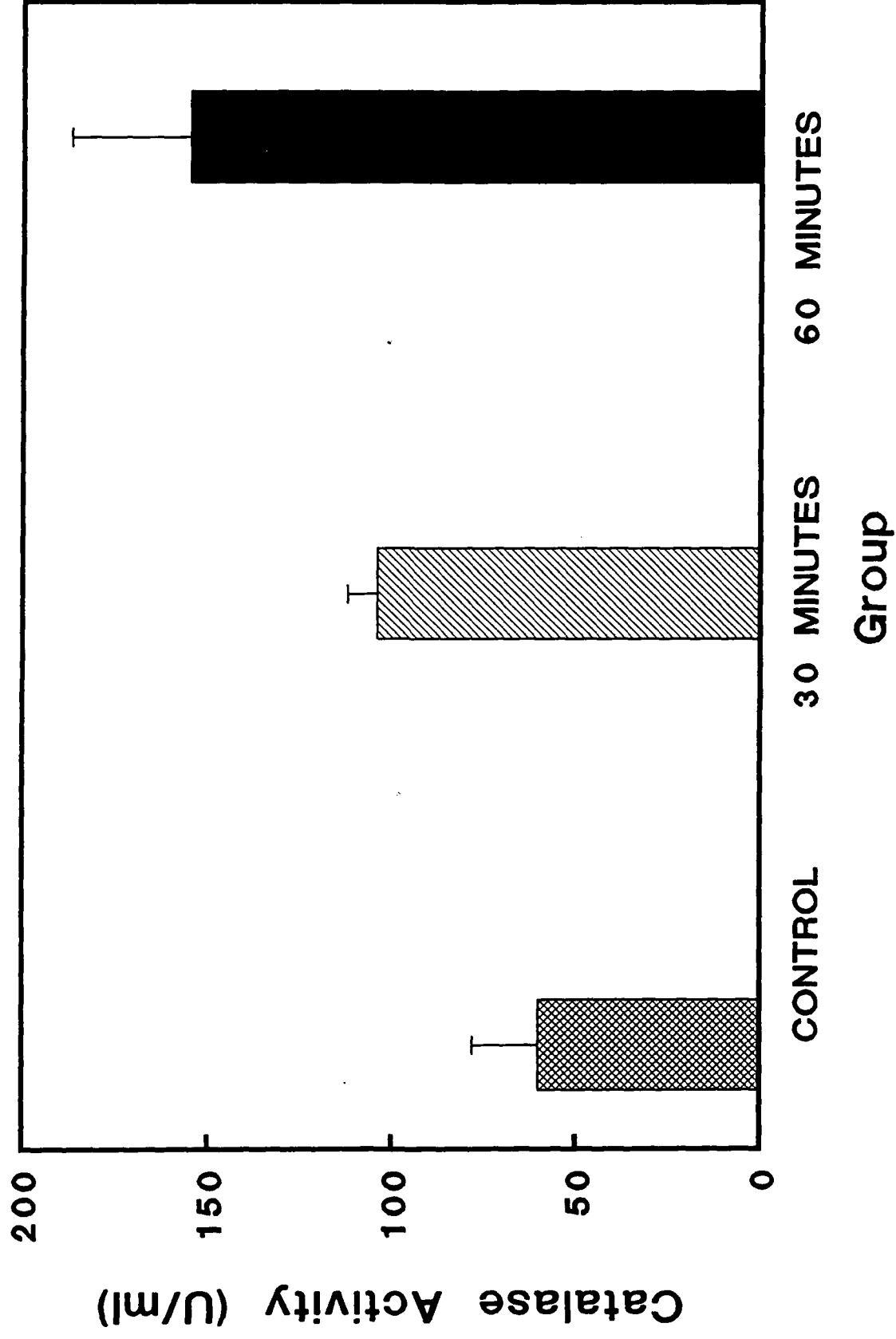


Figure 20

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